REMARKS

Claims 1-6 remain pending. Favorable reconsideration is respectfully requested.

The present invention relates to a method for detecting negatively supercoiled DNA *in cells*, characterized by including the steps of incorporating biotinylated psoralen into cells, irradiating the cells with long-wavelength UV rays, causing the cells to react with adivin which has been labeled with a color-developing substance, a fluorescent substance, or a chemiluminescent substance, and measuring developed color, emitted fluorescence, or emitted chemiluminescence *of the cells*. See Claim 1.

The present invention also relates to a method for detecting *a cell* containing negatively supercoiled DNA, characterized by including the steps of incorporating biotinylated psoralen into cells, irradiating the cells with long-wavelength UV rays, causing the cells to react with adivin which has been labeled with a color-developing substance, a fluorescent substance, or a chemiluminescent substance, and measuring developed color, emitted fluorescence, or emitted chemiluminescence *of the cells*. See Claim 2.

An important feature of the claimed methods is that the negatively supercoiled DNA is detected ***in cells***. Since the DNA is detected in the cells, the claimed methods do not require extracting the DNA from the cells in order to be analyzed.

The fact that the DNA does not have to be purified from the cells provides the claimed methods with a significant and surprising advantage as compared to methods that require the DNA to be extracted from cells prior to analysis. As discussed in the specification, the methods of the present invention are capable of detecting supercoiling in more loci in a genome as compared to methods which involve DNA purification and Southern hybridization. . See pages 20-21 of the specification. This striking result is also discussed in Matsumoto et al., *Journal of Cell Science*, 117 (17), 2004, an article published

by the Inventors of the present application after the present application was filed. In particular, the present specification states at pages 20-21:

> Through a conventional method employing the Southern hybridization, negative supercoils has been detected in only two regions of the entire genome (Non-Patent Document 4). Therefore, we conjectured that psoralen signals would be detected in only several regions of the genome. However, quite unexpectedly, many psoralen signals were observed in the salivary gland chromosomes. Such signals were detected in many interbands or puffs in which transcription was activated, but not detected in every interband or puff. When nicks had been introduced into DNA before crosslinking, psoralen signals were not detected. *Thus, the present invention is the first to visualize negatively supercoiled DNA on interphase chromosomes*. [Emphasis added.]

The rejection of the claims under 35 U.S.C. §103(a) over Sinden et al. in view of Saffrin et al. and Chevalier et al. is respectfully traversed. Those references fail to suggest the claimed methods.

Sinden et al. describe a method for assaying DNA supercoiling and topological domain size using trimethyl psoralen (see the Abstract and Table 1 at page 116). As described at page 117, second column, bottom, and in Table 1 at page 116, the DNA is purified before it is subjected to Southern hybridization. That is, the DNA is extracted from the cells before it is analyzed.

In contrast, as discussed above, the claimed methods do not specify purification of the DNA prior to analysis. Sinden et al. fail to suggest that the DNA does not have to be purified prior to analysis. In fact, that reference describes exactly the opposite!

Saffran et al. describe biotinylated psoralens (see the Abstract). Chevalier et al. reviews the use of biotin and digoxigenin as labels for hybridization probes. Neither of those references suggests that using biotinylated psoralen as claimed would allow negative DNA supercoiling to be in cells without disruption of the cells and subsequent purification of DNA.

In view of the foregoing, the claimed methods are not obvious over the combination of Sinden et al. in view of Saffrin et al. and Chevalier et al. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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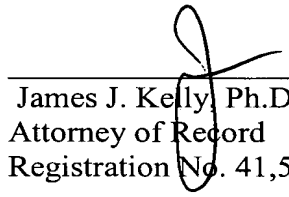

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